

# THE LANCET

## Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed.  
We post it as supplied by the authors.

Supplement to: Staedke SG, Gonahasa S, Dorsey G, et al. Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): a pragmatic, cluster-randomised trial embedded in a national LLIN distribution campaign. *Lancet* 2020; **395**: 1292–303.

## Supplemental file 1

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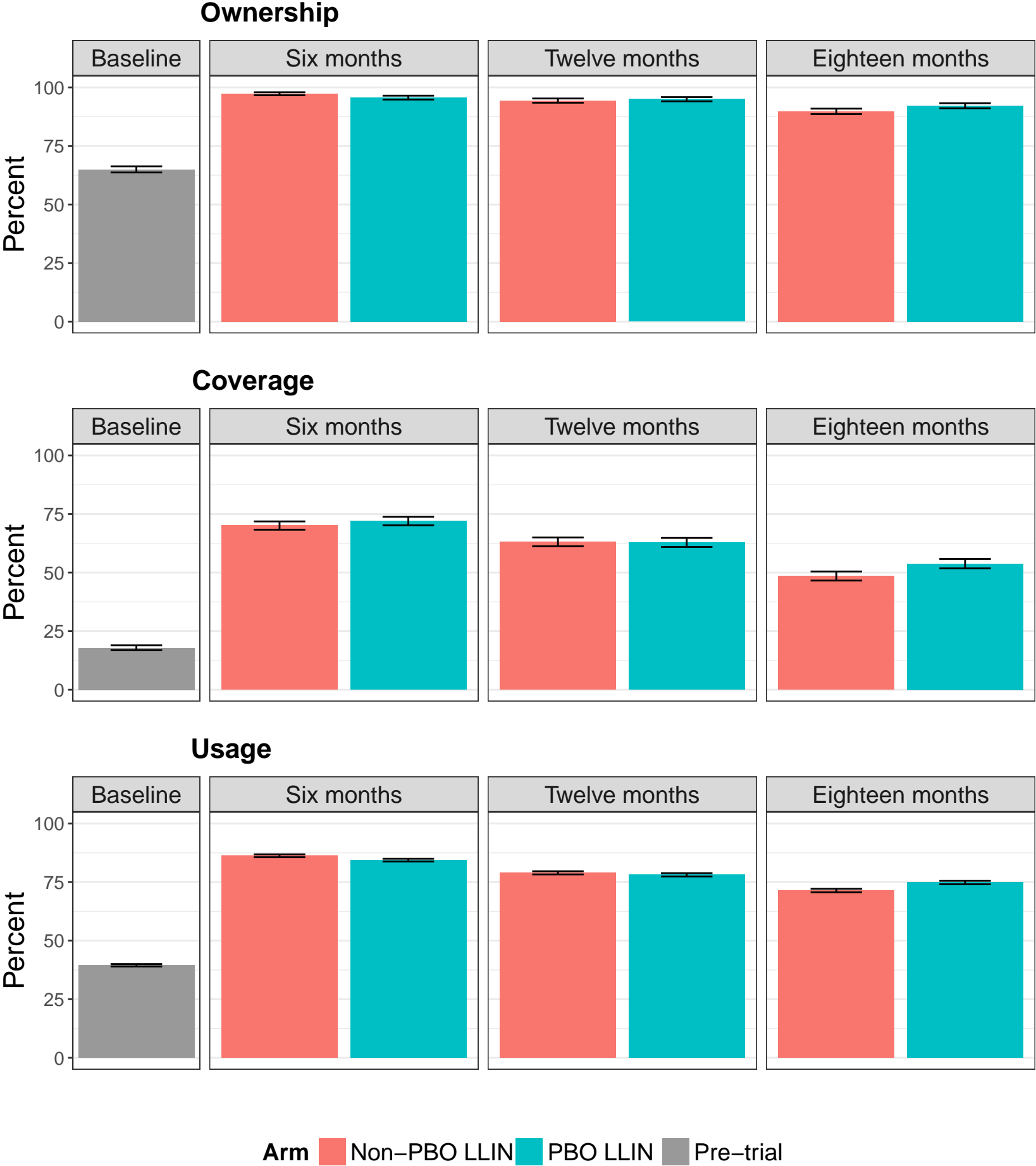
### High-performance liquid chromatography

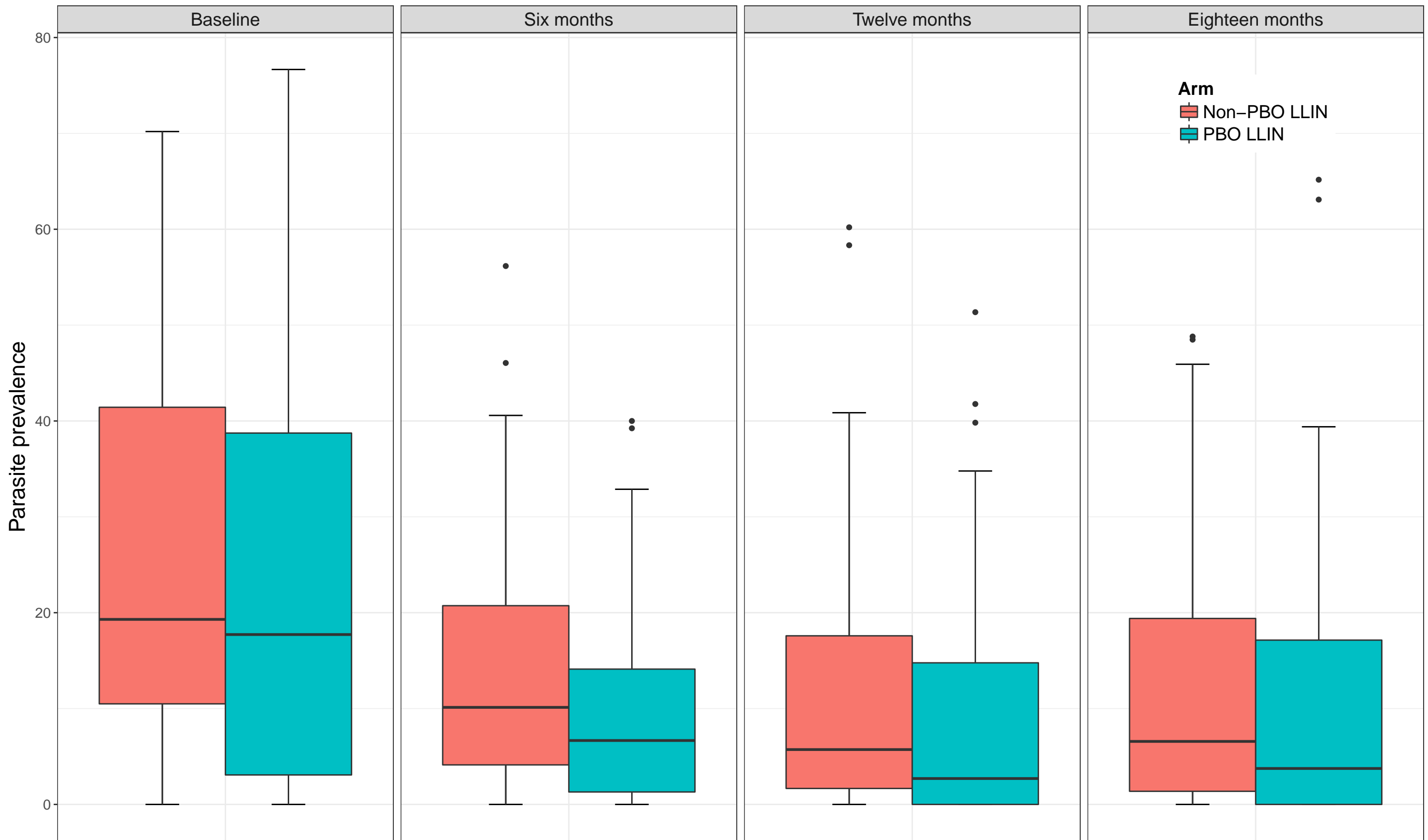
Two 30cm<sup>2</sup> net samples were cut from the top surface using an acrylic template. Five circular sections were cut from each LLIN sample using a die cutter, giving a total net area of approximately 40cm<sup>2</sup>. The weight of each LLIN sample was recorded. Samples were extracted in five ml of 10% 1-propanol in heptane with 0.1mg/ml dicyclohexyl phthalate (DCP) (Sigma Aldrich, UK) as an internal standard. Samples were boiled for 45min at 85°C, vortexing every 15min for ten seconds in a sealed 10ml glass tube. One ml of the insecticide extract was transferred to a clean glass tube and evaporated at 60°C. Samples were re-suspended in one ml of acetonitrile, and subsequently vortexed for one minute. Samples were filtered to remove impurities using a Chromacol syringe filter, 17mm 0.2µm pore PTFE (Thermo Scientific, UK) and 100µl of the filtrate was transferred to a Chromacol 300µl glass vial (Thermo Scientific, UK).

HPLC analysis was performed by injecting 10-µL aliquots of the extract on a reverse-phase Hypersil GOLD C18 column (75 Å, 250 × 4.6 mm, 5-µm particle size; Thermo Scientific, UK). To separate pyrethroids, PBO and DCP, a mobile phase of acetonitrile/water (70/30 v/v) was used at a flow rate of 1 ml/min. Detection of chromatographic peaks was carried out at a wavelength of 226 nm on an Agilent 1100 Series HPLC System; OpenLAB software V2.1 software (Agilent) was used for analysis. The quantities of pyrethroid and PBO were calculated in grams per kilogram of net material from standard curves established with known concentrations of authenticated standards for PBO, permethrin and deltamethrin (PESTANAL®, analytical standard, Sigma-Aldrich, UK) and corrected against internal standard (DCP) readings.

**Supplemental file 2: Long-lasting insecticidal net (LLIN) ownership, adequate coverage, and usage (at baseline, 6, 12, and 18 months).**

LLIN ownership: the proportion of households that owned at least one LLIN. Adequate LLIN coverage: the proportion of households that owned at least one LLIN for every two occupants. LLIN use: the proportion of household residents who slept under an LLIN the previous night.





**Supplemental file 3.** Trends in parasite prevalence in children aged 2-10 years as measured by microscopy (at baseline, 6, 12 & 18 months). LLIN=long-lasting insecticidal net. PBO=piperonyl butoxide.

## Supplemental file 4

**Table. Efficacy analysis of parasite prevalence stratified by region**

Duration following the intervention	Area/region	Study arm	N clusters ITT	Intention-to-treat analysis			As-treated analysis		
				n/N (%)	PR (95% CI)	p-value	n/N (%)	PR (95% CI)	p-value
6 months	East	Non PBO	17	313/1370 (23%)	reference	-	317/1427 (22%)	reference	-
		PBO	21	278/1647 (17%)	0.74 (0.59-0.91)	0.0056	247/1506 (16%)	0.66 (0.53-0.82)	0.0001
	West	Non PBO	35	239/2497 (10%)	reference	-	239/2417 (10%)	reference	-
		PBO	31	140/2151 (7%)	0.82 (0.63-1.07)	0.14	139/2108 (7%)	0.84 (0.65-1.10)	0.21
12 months	East	Non PBO	17	303/1384 (22%)	reference	-	311/1455 (21%)	reference	-
		PBO	21	330/1785 (19%)	0.80 (0.66-0.97)	0.021	297/1633 (18%)	0.69 (0.58-0.82)	<0.0001
	West	Non PBO	35	183/2407 (8%)	reference	-	182/2347 (8%)	reference	-
		PBO	31	97/2133 (5%)	0.75 (0.53-1.05)	0.090	95/2069 (5%)	0.76 (0.54-1.07)	0.12
18 months	East	Non PBO	17	286/1403 (20%)	reference	-	301/1466 (21%)	reference	-
		PBO	21	384/1681 (23%)	1.15 (0.94-1.39)	0.17	350/1547 (23%)	0.98 (0.82-1.18)	0.85
	West	Non PBO	35	258/2577 (10%)	reference	-	257/2510 (10%)	reference	-
		PBO	31	90/2234 (4%)	0.55 (0.41-0.73)	<0.0001	87/2161 (4%)	0.56 (0.42-0.74)	<0.0001

## Supplemental file 5

**Table. Efficacy analysis of moderate to severe anaemia (haemoglobin < 10 gm/dL) prevalence**

Duration following the intervention	Study arm	Intention-to-treat analysis			As-treated analysis		
		n/N (%)	PR (95% CI)	p-value	n/N (%)	PR (95% CI)	p-value
6 months	Non PBO LLIN	132/1639 (8%)	reference	-	138/1647 (8%)	reference	-
	PBO LLIN	97/1609 (6%)	0.76 (0.49-1.19)	0.23	90/1509 (6%)	0.71 (0.46-1.11)	0.14
12 months	Non PBO LLIN	151/1691 (9%)	reference	-	159/1692 (9%)	reference	-
	PBO LLIN	125/1681 (7%)	0.89 (0.53-1.49)	0.65	115/1584 (7%)	0.79 (0.47-1.33)	0.38
18 months	Non PBO LLIN	154/1657 (9%)	reference	-	155/1671 (9%)	reference	-
	PBO LLIN	110/1704 (6%)	0.72 (0.37-1.43)	0.36	107/1607 (7%)	0.73 (0.37-1.45)	0.37
6 months	PermaNet 2.0	99/1193 (8%)	reference	-	107/1259 (9%)	reference	-
	PermaNet 3.0	77/1072 (7%)	0.95 (0.58-1.55)	0.83	73/1035 (7%)	0.85 (0.52-1.40)	0.52
12 months	PermaNet 2.0	129/1223 (11%)	reference	-	138/1282 (11%)	reference	-
	PermaNet 3.0	95/1101 (9%)	0.90 (0.50-1.64)	0.74	87/1060 (8%)	0.81 (0.45-1.46)	0.49
18 months	PermaNet 2.0	92/1211 (8%)	reference	-	94/1281 (7%)	reference	-
	PermaNet 3.0	83/1135 (7%)	1.08 (0.58-2.01)	0.80	81/1092 (7%)	1.06 (0.58-1.96)	0.84
6 months	Olyset Net	33/446 (7%)	reference	-	31/388 (8%)	reference	-
	Olyset Plus	20/537 (4%)	0.51 (0.24-1.09)	0.081	17/474 (4%)	0.45 (0.20-1.02)	0.055
12 months	Olyset Net	22/468 (5%)	reference	-	21/410 (5%)	reference	-
	Olyset Plus	30/580 (5%)	1.10 (0.43-2.81)	0.84	28/524 (5%)	1.03 (0.39-2.73)	0.96
18 months	Olyset Net	62/446 (14%)	reference	-	61/390 (16%)	reference	-
	Olyset Plus	27/569 (5%)	0.36 (0.08-1.68)	0.19	26/515 (5%)	0.33 (0.07-1.58)	0.17